63. (New) The kit of Claim 62, further comprising a host cell deficient in said active reporter protein.

64. (New) The kit of Claim 62, wherein at least one of said first and second chimeric genes further contains a nucleotide sequence encoding an amino acid sequence capable of enabling at least one of the expressed first or second fusion proteins to at least partially anchor to the cell membrane of the host cell.

Please amend Claim 1 as follows:

1. (Amended) The method of Claim 61, wherein at least one of said first and second chimeric genes further contains a nucleotide sequence encoding an amino acid sequence capable of enabling at least one of the expressed first or second fusion proteins to at least partially anchor to the cell membrane of the host cell.

REMARKS

A preliminary amendment was previously submitted on September 16, 2002, replacing the claims originally included with the filing of the instant application. Applicants now wish to submit additional claims and make minor amendments to the claims submitted in the preliminary amendment dated September 16, 2002.

A clean copy of all pending claims as a result of the preliminary amendments is attached as Appendix A. A mark-up copy showing the present claim amendment is also attached as Appendix B.

According to 37 CFR § 1.115 (a), preliminary amendments may be entered in a patent application before the mail date of the first Office action from the PTO. Because a first Office action has not yet been received from the PTO, and because the nature of the changes contained in the present preliminary amendment would require no additional effort on behalf of the PTO to conduct prior art searches, applicants submit that the changes contained herein do not unduly interfere with the preparation of a first Office action, and should therefore be entered.

In view of the foregoing, applicants submit that this application is in condition for allowance. An early notice of such allowance is respectfully requested.

Respectfully submitted,

Date: 12/9/02

Jay Z. Zhang
Attorney for Applicants
Registration No. 44,003

Intellectual Property Department
Myriad Genetics, Inc.
320 Wakara Way
Salt Lake City, UT 84108
Telephone: (801) 584-3600
Facsimile: (801) 584-3640

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Michael Moreno

12-9-02
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APPENDIX A

FINAL PENDING CLAIMS FOR EXAMINATION

1. The method of Claim 61, wherein at least one of said first and second chimeric genes further contains a nucleotide sequence encoding an amino acid sequence capable of enabling at least one of the expressed first or second fusion proteins to at least partially anchor to the cell membrane of the host cell.

2. The method of Claim 1, wherein said first inactive reporter polypeptide is an N-terminal fragment of said active reporter protein and said second inactive reporter polypeptide is the remaining C-terminal fragment of said active reporter protein.

3. The method of Claim 1, wherein said host cell is a diploid cell and said step of introducing into said host cell said first chimeric gene and said second chimeric gene comprises mating a first haploid yeast cell having said first chimeric gene with a second haploid yeast cell having said second chimeric gene.

4. The method of Claim 1, wherein said active reporter protein is detectable by a color assay.

5. The method of Claim 1, wherein said active reporter protein is an auxotrophic protein and is detectable by a cell viability assay.

6. The method of Claim 1, wherein said first inactive reporter polypeptide is fused to the N-terminus of said N-intein.

7. The method of Claim 1, wherein said second inactive reporter polypeptide is fused to the C-terminus of said C-intein.

8. The method of Claim 1, wherein one of said first or second test polypeptides is at least partially anchored to the cell membrane while the other is contained inside the cell.

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9. The method of Claim 1, wherein both said first and second test polypeptides are at least partially anchored to the cell membrane.

10. A method for detecting protein-protein interaction between a first test polypeptide and a second test polypeptide, comprising:

expressing a first fusion protein and a second fusion protein in a host cell, said first fusion protein having said first test polypeptide, an N-intein, and a first inactive reporter polypeptide fused to the N-terminus of an N-intein, said second fusion protein having said second test polypeptide, a C-intein, and a second inactive reporter polypeptide fused to the C-terminus of said C-intein, wherein ligation between the C-terminus of said first inactive reporter polypeptide and the N-terminus of said second inactive reporter polypeptide forms an active reporter protein, wherein said first and second fusion proteins further contain an amino acid sequence capable of enabling the expressed first and second fusion proteins to at least partially anchor to the cell membrane of the host cell, and wherein said first and second test polypeptides at least partially reside outside the cell; and

detecting said active reporter protein.

11. The method of Claim 10, wherein said first inactive reporter polypeptide is an N-terminal fragment of said active reporter protein and said second inactive reporter polypeptide is the remaining C-terminal fragment of said active reporter protein.

12. The method of Claim 10, wherein said active reporter protein is detectable by a color assay.

13. The method of Claim 12, wherein said active reporter protein is selected from the group consisting of β-galactosidase, luciferase, green fluorescence protein, blue fluorescence protein, alkaline phosphatase, and horseradish peroxidase.

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14. The method of Claim 10, wherein said active reporter protein is an auxotrophic protein and is detectable by a cell viability assay.

15. The method of Claim 14, wherein the host cell is a yeast cell deficient in *URA3* gene, and wherein the first inactive reporter is an N-terminal portion of orotidine-5'-phosphate decarboxylase and the second inactive reporter is a C-terminal portion of orotidine-5'-phosphate decarboxylase.

16. A method for detecting protein-protein interaction between a first test polypeptide and a second test polypeptide, comprising:

introducing into a host cell a first chimeric gene and a second chimeric gene, said first chimeric gene encoding a first fusion protein having said first test polypeptide, an N-intein, and a first inactive reporter polypeptide fused to the N-terminus of an N-intein, said second chimeric gene encoding a second fusion protein having said second test polypeptide, a C-intein, and a second inactive reporter polypeptide fused to the C-terminus of said C-intein, wherein ligation between the C-terminus of said first inactive reporter polypeptide and the N-terminus of said second inactive reporter polypeptide forms an active reporter protein, wherein said first and second chimeric genes further contain a nucleotide sequence encoding an amino acid sequence capable of enabling the expressed first and second fusion proteins to anchor to the cell membrane of the host cell such that said first and second test polypeptides are exposed outside the cell while the inactive reporter polypeptides and the N-intein and C-intein are retained within the cell;

expressing said first fusion protein and said second fusion protein in said host cell; and

detecting said active reporter protein.

17. The method of Claim 16, wherein said first inactive reporter polypeptide is an N-terminal fragment of said active reporter protein and said second inactive reporter polypeptide is the remaining C-terminal fragment of said active reporter protein.

18. The method of Claim 16, wherein said host cell is a diploid yeast cell and said step of introducing into said host cell said first chimeric gene and said second chimeric gene

comprises mating a first haploid yeast cell having said first chimeric gene with a second haploid yeast cell having said second chimeric gene.

19. The method of Claim 16, wherein said first test polypeptide is fused to the N-terminus of a first transmembrane domain which is fused to the N-terminus of said first inactive reporter polypeptide that is fused to the N-terminus of said N-intein in said first fusion protein.

20. The method of Claim 16, wherein said second test polypeptide is fused to the N-terminus of a second transmembrane domain which is fused to the N-terminus of said C-intein that is fused to the N-terminus of said second inactive reporter in said second fusion protein.

21. The method of Claim 16, wherein said active reporter protein is detectable by a color assay.

22. The method of Claim 21, wherein said active reporter protein is selected from the group consisting of β-galactosidase, luciferase, green fluorescence protein, blue fluorescence protein, alkaline phosphatase, horseradish peroxidase, and derivatives thereof.

23. The method of Claim 16, wherein said active reporter protein is an auxotrophic protein and is detectable by a cell viability assay.

24. The method of Claim 23, wherein the host cell is a yeast cell deficient in *URA3* gene, and wherein the first inactive reporter is an N-terminal portion of orotidine-5'-phosphate decarboxylase and the second inactive reporter is a C-terminal portion of orotidine-5'-phosphate decarboxylase.

25. A method for detecting protein-protein interaction between a first test polypeptide and a second test polypeptide, comprising:

introducing into a host cell a first chimeric gene and a second chimeric gene, said first chimeric gene encoding a first fusion protein having a first test polypeptide fused to the N-

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terminus of a first transmembrane domain which is fused to the N-terminus of a first inactive reporter polypeptide that is fused to the N-terminus of an N-intein, said second chimeric gene encoding a second fusion protein having a second test polypeptide fused to the N-terminus of a second transmembrane domain which is fused to the N-terminus of a C-intein that is fused to the N-terminus of a second inactive reporter,

wherein when expressed in said host cell said first and second fusion proteins are anchored to the cell membrane of the host cell with said first and second test polypeptides being exposed to the outside the cell, and the inactive reporters, the N-intein and C-intein all being retained within the cell,

wherein ligation between the C-terminus of said first inactive reporter polypeptide and the N-terminus of said second inactive reporter polypeptide forms an active reporter protein, and

wherein said host cell lacks said active reporter protein;

expressing said first fusion protein and said second fusion protein in said host cell; and

detecting said active reporter protein.

26. A method for detecting protein-protein interaction, comprising:

providing a prey fusion protein expression library comprising a plurality of chimeric genes contained in a plurality of prey haploid yeast cells of a first mating type, wherein each of said plurality of chimeric genes encodes a fusion protein containing a prey test polypeptide, an N-intein, a first inactive reporter polypeptide;

providing a plurality of bait haploid yeast cells having a mating type opposite to that of said prey haploid yeast cells, said bait haploid yeast cells expressing a bait fusion protein containing a bait test polypeptide, a C-intein, a second inactive reporter polypeptide, and an amino acid sequence which enables the bait fusion protein to anchor to the cell membrane of the bait haploid yeast cell, wherein ligation between said first inactive reporter polypeptide and said second inactive reporter polypeptide forms an active reporter protein;

mating said plurality of bait haploid yeast cells and said plurality of prey haploid yeast cells to form a plurality of diploid yeast cells; and

detecting said active reporter protein in said plurality of diploid yeast cells.

APPENDIX A

27. The method of Claim 26, wherein said prey test polypeptide is in the cytoplasm of said diploid yeast cells.

28. The method of Claim 26, wherein said prey test polypeptide is at least partially exposed outside said diploid yeast cells.

29. The method of Claim 26, wherein said prey test polypeptide at least partially resides in the cell membrane of said diploid yeast cells.

30. The method of Claim 26, wherein said prey test polypeptide is fused to the N-terminus of a transmembrane domain which is fused to the N-terminus of said first inactive reporter polypeptide that is fused to the N-terminus of said N-intein in said prey fusion protein, and wherein said bait test polypeptide is fused to the N-terminus of a transmembrane domain which is fused to the N-terminus of said C-intein that is fused to the N-terminus of said second inactive reporter in said bait fusion protein.

31. A method for detecting protein-protein interaction, comprising:

providing a prey fusion protein expression library comprising a plurality of chimeric genes contained in a plurality of prey haploid yeast cells of a first mating type, wherein each of said plurality of chimeric genes encodes a fusion protein containing a prey test polypeptide, a C-intein, a first inactive reporter polypeptide;

providing a plurality of bait haploid yeast cells having a mating type opposite to that of said prey haploid yeast cells, said bait haploid yeast cells expressing a bait fusion protein containing a bait test polypeptide, an N-intein, a second inactive reporter polypeptide, and an amino acid sequence which enables the bait fusion protein to anchor to the cell membrane of the bait haploid yeast cell, wherein ligation between said first inactive reporter polypeptide and said second inactive reporter polypeptide forms an active reporter protein;

mating said plurality of bait haploid yeast cells and said plurality of prey haploid yeast cells to form a plurality of diploid yeast cells; and

detecting said active reporter protein in said plurality of diploid yeast cells.

32. The method of Claim 31, wherein said prey test polypeptide is in the cytoplasm of said diploid yeast cells.

33. The method of Claim 31, wherein said prey test polypeptide is at least partially exposed outside said diploid yeast cells.

34. The method of Claim 31, wherein said prey test polypeptide at least partially resides in the membrane of said diploid yeast cells.

35. The method of Claim 30, wherein said prey test polypeptide is fused to the N-terminus of a transmembrane domain which is fused to the N-terminus of said C-intein that is fused to the N-terminus of said first inactive reporter polypeptide in said prey fusion protein, and wherein said bait test polypeptide is fused to the N-terminus of a transmembrane domain which is fused to the N-terminus of said second inactive reporter that is fused to the N-terminus of said N-intein in said bait fusion protein.

36. A method for selecting compounds capable of modulating a protein-protein interaction between a first test polypeptide and a second test polypeptide, comprising:

introducing into a host cell a first chimeric gene and a second chimeric gene, said first chimeric gene encoding a first fusion protein having said first test polypeptide, an N-intein, and a first inactive reporter polypeptide, said second chimeric gene encoding a second fusion protein having said second test polypeptide, a C-intein, and a second inactive reporter polypeptide, wherein ligation between the said first inactive reporter polypeptide and said second inactive reporter polypeptide forms an active reporter protein, wherein at least one of said first and second chimeric genes further contains a nucleotide sequence encoding an amino acid sequence capable of enabling at least one of the expressed first or second fusion proteins to at least partially anchor to the cell membrane of the host cell;

expressing said first fusion protein and said second fusion protein in said host cell in the presence of one or more test compounds; and

detecting said active reporter protein.

37. The method of Claim 36, wherein said first inactive reporter polypeptide is an N-terminal fragment of said active reporter protein and said second inactive reporter polypeptide is the remaining C-terminal fragment of said active reporter protein.

38. The method of Claim 36, wherein said host cell is a diploid cell and said step of introducing into said host cell said first chimeric gene and said second chimeric gene comprises mating a first haploid yeast cell having said first chimeric gene with a second haploid yeast cell having said second chimeric gene.

39. The method of Claim 36, wherein said active reporter protein is detectable by a color assay.

40. The method of Claim 36, wherein said active reporter protein is an auxotrophic protein and is detectable by a cell viability assay.

41. The method of Claim 36, wherein said first inactive reporter polypeptide is fused to the N-terminus of said N-intein.

42. The method of Claim 36, wherein said second inactive reporter polypeptide is fused to the C-terminus of said C-intein.

43. The method of Claim 36, wherein one of said first or second test polypeptides is at least partially anchored to the cell membrane while the other is contained inside the cell.

44. The method of Claim 36, wherein both said first and second test polypeptides are at least partially anchored to the cell membrane.

APPENDIX A

45. A method for selecting compounds capable of interfering with a protein-protein interaction between a first test polypeptide and a second test polypeptide, comprising:

expressing a first fusion protein and a second fusion protein in a host cell in the presence of one or more test compounds, wherein said first fusion protein contains said first test polypeptide, an N-intein, and a first inactive reporter polypeptide fused to the N-terminus of said N-intein, said second fusion protein contains said second test polypeptide, a C-intein, and a second inactive reporter polypeptide fused to the C-terminus of said C-intein, wherein ligation between the C-terminus of said first inactive reporter polypeptide and the N-terminus of said second inactive reporter polypeptide forms an active reporter protein, wherein said first and second fusion proteins further contain an amino acid sequence capable of enabling the expressed first and second fusion proteins to at least partially anchor to the cell membrane of the host cell, and wherein said first and second test polypeptides at least partially reside outside the cell; and

detecting said active reporter protein.

46. The method of Claim 45, wherein said first inactive reporter polypeptide is an N-terminal fragment of said active reporter protein and said second inactive reporter polypeptide is the remaining C-terminal fragment of said active reporter protein.

47. The method of Claim 45, wherein said active reporter protein is detectable by a color assay.

48. The method of Claim 47, wherein said active reporter protein is selected from the group consisting of β-galactosidase, luciferase, green fluorescence protein, blue fluorescence protein, alkaline phosphatase, and horseradish peroxidase.

49. The method of Claim 45, wherein said active reporter protein is an auxotrophic protein and is detectable by a cell viability assay.

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50. The method of Claim 49, wherein the host cell is a yeast cell deficient in *URA3* gene, and wherein the first inactive reporter is an N-terminal portion of orotidine-5'-phosphate decarboxylase and the second inactive reporter is a C-terminal portion of orotidine-5'-phosphate decarboxylase.

51. A method for selecting compounds capable of interfering with a protein-protein interaction between a first test polypeptide and a second test polypeptide, comprising:

introducing into an yeast cell a first chimeric gene and a second chimeric gene, said first chimeric gene encoding a first fusion protein having said first test polypeptide, an N-intein, and a first inactive reporter polypeptide fused to the N-terminus of said N-intein, said second chimeric gene encoding a second fusion protein having said second test polypeptide, a C-intein, and a second inactive reporter polypeptide fused to the C-terminus of said C-intein, wherein ligation between the C-terminus of said first inactive reporter polypeptide and the N-terminus of said second inactive reporter polypeptide forms an active reporter protein, wherein said first and second chimeric genes further contain a nucleotide sequence encoding an amino acid sequence capable of enabling the expressed first and second fusion proteins to anchor to the cell membrane of the host cell, with said first and second test polypeptides being exposed outside the cell while the inactive reporters and the N-intein and C-intein being retained within the cell;

expressing said first fusion protein and said second fusion protein in said yeast cell in the presence of one or more test compounds; and

detecting said active reporter protein.

52. The method of Claim 51, wherein said first inactive reporter polypeptide is an N-terminal fragment of said active reporter protein and said second inactive reporter polypeptide is the remaining C-terminal fragment of said active reporter protein.

53. The method of Claim 51, wherein said host cell is a diploid yeast cell and said step of introducing into said host cell said first chimeric gene and said second chimeric gene comprises mating a first haploid yeast cell having said first chimeric gene with a second haploid yeast cell having said second chimeric gene.

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54. The method of Claim 51, wherein said first test polypeptide is fused to the N-terminus of a first transmembrane domain which is fused to the N-terminus of said first inactive reporter polypeptide that is fused to the N-terminus of said N-intein in said first fusion protein.

55. The method of Claim 51, wherein said second test polypeptide is fused to the N-terminus of a second transmembrane domain which is fused to the N-terminus of said C-intein that is fused to the N-terminus of said second inactive reporter in said second fusion protein.

56. The method of Claim 51, wherein said active reporter protein is detectable by a color assay.

57. The method of Claim 56, wherein said active reporter protein is selected from the group consisting of β-galactosidase, luciferase, green fluorescence protein, blue fluorescence protein, alkaline phosphatase, and horseradish peroxidase.

58. The method of Claim 57, wherein said active reporter protein is an auxotrophic protein and is detectable by a cell viability assay.

59. The method of Claim 58, wherein the host cell is a yeast cell deficient in *URA3* gene, and wherein the first inactive reporter is an N-terminal portion of orotidine-5'-phosphate decarboxylase and the second inactive reporter is a C-terminal portion of orotidine-5'-phosphate decarboxylase.

60. A method for detecting protein-protein interaction between a first test polypeptide and a second test polypeptide, comprising:

producing in a host cell a first fusion protein and a second fusion protein, said first fusion protein having said first test polypeptide and an N-intein, said second fusion protein having said second test polypeptide and a C-intein, wherein at least one of the two fusion proteins has an

inactive reporter capable of being converted to an active reporter protein upon trans-splicing through said N-intein and said C-intein; and

determining the production of said active reporter protein.

61. A method for detecting protein-protein interaction between a first test polypeptide and a second test polypeptide, comprising:

introducing into a host cell a first chimeric gene and a second chimeric gene, said first chimeric gene encoding a first fusion protein having said first test polypeptide, an N-intein, and a first inactive reporter polypeptide, said second chimeric gene encoding a second fusion protein having said second test polypeptide, a C-intein, and a second inactive reporter polypeptide, wherein ligation between said first inactive reporter polypeptide and said second inactive reporter polypeptide forms an active reporter protein;

expressing said first fusion protein and said second fusion protein in said host cell; and

detecting said active reporter protein.

62. A kit comprising:

a first vector containing a first chimeric gene encoding a first inactive reporter polypeptide fused to the N-terminus of an N-intein and containing an operably linked first multiple cloning site (MCS) such that when a nucleic acid encoding a first test polypeptide is inserted into said first multiple cloning site, said first chimeric gene is capable of expressing a first fusion protein containing said N-intein, said first test polypeptide, and said first inactive reporter polypeptide fused to the N-terminus of said N-intein;

a second vector containing a second chimeric gene encoding a second inactive reporter polypeptide fused to the C-terminus of a C-intein and containing an operably linked second multiple cloning site (MCS) such that when a nucleic acid encoding a second test polypeptide is inserted into said second multiple cloning site, said second chimeric gene is capable of expressing a second fusion protein containing said C-intein, said second test polypeptide, and said second inactive reporter polypeptide fused to the C-terminus of said C-intein, wherein ligation between the C-terminus of said first inactive reporter polypeptide and the N-terminus of said second inactive reporter polypeptide forms an active reporter protein; and

instructions for using said first and second vectors in detecting protein-protein interactions.

63. The kit of Claim 62, further comprising a host cell deficient in said active reporter protein.

64. The kit of Claim 62, wherein at least one of said first and second chimeric genes further contains a nucleotide sequence encoding an amino acid sequence capable of enabling at least one of the expressed first or second fusion proteins to at least partially anchor to the cell membrane of the host cell.

APPENDIX B
Mark-up Copy of the Amended Claims

Please add the following new Claims 60-64:

60. (New) A method for detecting protein-protein interaction between a first test polypeptide and a second test polypeptide, comprising:

producing in a host cell a first fusion protein and a second fusion protein, said first fusion protein having said first test polypeptide and an N-intein, said second fusion protein having said second test polypeptide and a C-intein, wherein at least one of the two fusion proteins has an inactive reporter capable of being converted to an active reporter protein upon trans-splicing through said N-intein and said C-intein; and

determining the production of said active reporter protein.

61. (New) A method for detecting protein-protein interaction between a first test polypeptide and a second test polypeptide, comprising:

introducing into a host cell a first chimeric gene and a second chimeric gene, said first chimeric gene encoding a first fusion protein having said first test polypeptide, an N-intein, and a first inactive reporter polypeptide, said second chimeric gene encoding a second fusion protein having said second test polypeptide, a C-intein, and a second inactive reporter polypeptide, wherein ligation between said first inactive reporter polypeptide and said second inactive reporter polypeptide forms an active reporter protein;

expressing said first fusion protein and said second fusion protein in said host cell; and

detecting said active reporter protein.

62. (New) A kit comprising:

a first vector containing a first chimeric gene encoding a first inactive reporter polypeptide fused to the N-terminus of an N-intein and containing an operably linked first multiple cloning site (MCS) such that when a nucleic acid encoding a first test polypeptide is inserted into said first multiple cloning site, said first chimeric gene is capable of expressing a first fusion protein containing said N-intein, said first test polypeptide, and said first inactive reporter polypeptide fused to the N-terminus of said N-intein;

APPENDIX B
Mark-up Copy of the Amended Claims

a second vector containing a second chimeric gene encoding a second inactive reporter polypeptide fused to the C-terminus of a C-intein and containing an operably linked second multiple cloning site (MCS) such that when a nucleic acid encoding a second test polypeptide is inserted into said second multiple cloning site, said second chimeric gene is capable of expressing a second fusion protein containing said C-intein, said second test polypeptide, and said second inactive reporter polypeptide fused to the C-terminus of said C-intein, wherein ligation between the C-terminus of said first inactive reporter polypeptide and the N-terminus of said second inactive reporter polypeptide forms an active reporter protein; and

instructions for using said first and second vectors in detecting protein-protein interactions.

63. (New) The kit of Claim 62, further comprising a host cell deficient in said active reporter protein.

64. (New) The kit of Claim 62, wherein at least one of said first and second chimeric genes further contains a nucleotide sequence encoding an amino acid sequence capable of enabling at least one of the expressed first or second fusion proteins to at least partially anchor to the cell membrane of the host cell.

Please amend Claim 1 as follows:

(Insertions appear as underlined, while deletions appear as double strikethrough text.)

1. (Amended) The method of Claim 61, ~~A method for detecting protein-protein interaction between a first test polypeptide and a second test polypeptide, comprising:~~

~~introducing into a host cell a first chimeric gene and a second chimeric gene, said first chimeric gene encoding a first fusion protein having said first test polypeptide, an N-intein, and a first inactive reporter polypeptide, said second chimeric gene encoding a second fusion protein having said second test polypeptide, a C-intein, and a second inactive reporter polypeptide, wherein ligation between said first inactive reporter polypeptide and said second inactive reporter polypeptide forms an active reporter protein,~~ wherein at least one of said first and second chimeric genes further contains a nucleotide sequence encoding an amino acid sequence

capable of enabling at least one of the expressed first or second fusion proteins to at least partially anchor to the cell membrane of the host cell;

~~expressing said first fusion protein and said second fusion protein in said host cell; and~~

~~detecting said active reporter protein~~.